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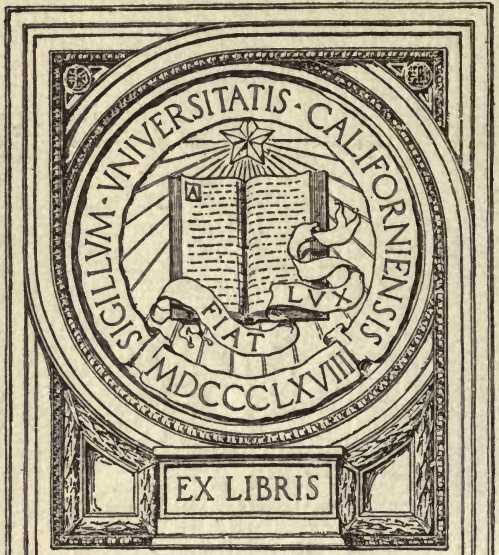

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RELATION OF THE COMMON MILK STREPTOCOCCI TO STREPTOCOCCUS PYOGENES

PRESENTED TO THE UNIVERSITY OF NEBRASKA

BY

CLARENCE EMERSON

AS A THESIS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

UNIVERSITY

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RELATION OF THE COMMON MILK STREPTOCOCCI TO STREPTOCOCCUS PYOGENES.

BY CLARENCE EMERSON.

Aside from the importance of this class of streptococci in milk, because of the chemical changes produced therein, there is a possibility that certain varieties may be of considerable interest because of their effect on the health of the consumer. The significance of streptococci in milk has been a matter of discussion for some time. It has long been known that these organisms are very often present in the milk from cows suffering from mastitis, and that, when freshly isolated from the diseased udders, they are pathogenic to lower animals. Formerly it was supposed that only milk from diseased udders contained streptococci, but more recent investigations have disclosed the fact that they are present in a large number of samples of normal milk from healthy cows. Indeed, the milk is rare which contains no organisms of this class. The recognition of their significance becomes especially desirable when we note the close cultural similarity of the strains isolated from milk to the common pathogenic varieties. Many investigators hold to the view that disease in man may result from the use of milk from cows suffering from mastitis. Some of these are mentioned in Bulletin No. 41 of the Hygienic Laboratory as follows:

"Holst,[b] Stokes and Wegefarth,[c] Beck,[d] Lameris and Von Harrevelt,[e] Kenwood,[f] Savage,[g] and many others saw epidemics of sore throat with swelling of the cervical glands, colic, diarrhea, and fever lasting several days, which were ascribed to the use of milk from cows with garget. Such milk when examined was found to contain pus and streptococci in great abundance. Holst in an experiment upon himself drank 200 cubic centimeters of a culture of a streptococcus isolated from such a milk during an outbreak, and became ill with colic and diarrhea.

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"Petruschky and Kriebel[h] and Holling[i] see in the streptococci found in milk a cause of summer diarrhea in children." [1]

It is not surprising that streptococci from an active disease process in the glands of the cow are able to exert a pathogenic influence when introduced into man and the lower animals. The significance of streptococci from such a source is not necessarily the significance of streptococci isolated from milk of normal, healthy udders. In the latter case we have a nonpathogenic germ, the exact relation of which to the pathogenic streptococci is not known. If these organisms are attenuated forms of the pathogenic streptococci, it is possible that under favorable conditions their pathogenicity may be restored and we may then attach to them considerable importance. The significance then of the common milk streptococci, so far as the health of the consumer is concerned, depends upon their relation to the streptococcus pyogenes.

PURPOSE OF THE INVESTIGATION.

This investigation was undertaken to determine the frequency of the occurrence of these streptococci in milk and their relation to streptococcus pyogenes.

METHOD OF INVESTIGATION.

The ordinary method of isolation by means of plate culture was employed in obtaining strains of the milk streptococci. They were then grown on gelatin, agar, potato, milk, and Löfflers blood-serum and comparison made with similar cultures of a strain of streptococcus pyogenes which was originally obtained from the Marine Hospital Service. Those showing cultural similarity to the latter organisms were retained for further study.

THE MILK STREPTOCOCCI.

The common lactic bacteria were formerly classified in three groups:

1. Bacterium Aerogenes.
2. Bacterium Acidi Lactici.
3. Streptococcus Lacticus.

In 1903 Kruse[2] pointed out the close cultural similarity of groups two and three and suggested that the oval bacillus was the result of rapid growth of the streptococcus in the process of division. In 1906 Heinemann[3] published the results of a careful comparison of bacillus acidi lactici with pathogenic, sewage and water streptococci. He showed that there was no constant differences in cultural characteristics or in their action on milk and contends that bacillus acidi lactici is non-existent, the organisms formerly classed as such being rapidly developing strains of streptococcus lacticus.

Concerning the resemblance of streptococcus lacticus to the streptococcus pyogenes, Paul G. Heinemann says: "Streptococcus lacticus agrees in morphological, cultural and coagulative properties with pathogenic, fecal and sewage streptococci."[4] Miller makes the following statement in this regard: "In view of the facts presented the assumption seems justified that the streptococcus pyogenes and the streptococcus lacticus, the common organisms of lactic-acid fermentation, are indistinguishable by our present methods."[5]

The strains of milk streptococci, commonly classed as streptococcus lacticus, are culturally similar to streptococcus pyogenes. So far as is known at the present time the only essential difference between these milk streptococci and the streptococcus pyogenes is the difference in pathogenicity. It might well be that these are but attenuated forms of the pathogenic germ and it has been one of the chief efforts in this investigation to develop virulency in a number of strains of this class.

FREQUENCY OF OCCURRENCE OF MILK STREPTOCOCCI.

Two methods of investigation were utilized to throw light on this question. First, direct examination of cover-glass spreads of milk stained with Löfflers methylene blue. This would give some idea of the number of streptococci as a class, but would not enable one to draw any conclusions as to the number of any particular variety.

By the stain method streptococci were demonstrated in

twenty-six out of thirty-four samples of milk examined. None but distinct chains of from six to eight or more elements were considered. Plate cultures were then made and the isolated streptococci were studied to determine what proportion of them were streptococcus lacticus.

The following results were obtained in attempts to isolate the germs by the plate method:

(+ = germ isolated. — = germ not isolated.)

1. +	7. —	13. +
2. —	8. +	14. —
3. +	9. —	15. +
4. +	10. +	16. —
5. +	11. —	17. +
6. —	12. +	18. —

I was able to isolate streptococci from ten out of eighteen samples of milk, whereas the stained preparations showed a larger proportion than that to be present. Cultural study of these strains indicated that of these ten, seven belonged to the streptococcus lacticus group, while the other three were classed with other varieties. It will be seen that a large per cent of the streptococci occurring in fresh milk belong to this class, which because of their cultural similarity are suspected of being related to the pathogenic streptococci.

RELATION OF STREPTOCOCCUS LACTICUS TO STREPTOCOCCUS PYOGENES.

A careful comparison of various strains of streptococcus lacticus on the ordinary culture media with similar cultures of streptococcus pyogenes afforded no means of differentiation. Their action on dextrose, lactose, maltose, glucose, saccharose galactose, mannit and inulin was studied without determining any constant differential features.

BLOOD AGAR.

Beside the ordinary cultures, I had hoped to throw some light on the relation of these germs to the streptococcus pyogenes by the use of blood agar, since it is claimed by many investigators

that the pathogenic varieties cause a much greater hemolysis than the nonpathogenic varieties.

Schottmüller,[6] in his investigations on the relative hemolytic action of various strains of streptococci, found that the virulent pathogenic streptococci cause abundant hemolysis, the streptococcus mucosus shows hemolytic action after many days and the pneumococcus shows only a narrow area of greenish hue surrounding the colony. Schlesinger[7] obtained no hemolysis with the nonpathogenic varieties, while the pathogenic strains possessed abundant hemolytic power. When the virulence of the germ was increased by passage through animals the hemolysis is increased.

In my experiments, I withdrew blood under sterile precautions from the jugular of a horse and used it for making blood agar plates. After solidifying, these plates were inoculated with the milk streptococci. In no instance did any of these organisms show any hemolysis. A contamination from the air in two of the plates produced a beautiful decoloration, this fact being mentioned to indicate that the media was favorable for this action. If we are able to draw any conclusions from these results, it must be that these streptococci are different from the common pathogenic varieties or else they are in such an attenuated condition that this property has been lost.

FILTERED BEEF-TEA CULTURES.

No attempt was made to grow these strains in filtered beef-tea cultures of the streptococcus because the results of various authors working on the streptococcus pyogenes present such conflicting results that one would not be able to draw any satisfactory conclusions from such an investigation.

NEUTRAL RED.

Gordon[8] has used neutral red for differentiation of streptococci. When grown under anaerobic conditions forty-eight hours at 37° he found the streptococcus brevus reacted positive, while streptococcus longus and pyogenes reacted negative. This, how-

ever, does not permit differentiation of the pyogenic class from other long streptococci.

COLLODIUM SAC EXPERIMENTS TO INCREASE VIRULENCE.

It will be noted that none of these varieties were virulent at the time of this study, further that the seven of my own isolation was obtained from milk of healthy udders. Four strains were sent to me by Mr. L. A. Rogers of the Bureau of Animal industry. (I have not been informed as to the origin of the four cultures sent by Mr. Rogers, except that they are milk streptococci.)

Experiments have proved that streptococci originally virulent may become nonvirulent after long cultivation on artificial media, and that they may return to their original properties after being passed through the bodies of susceptible animals. For this purpose, collodium sacs were prepared. These were filled with a beef-tea culture of the germ and placed in the abdominal cavities of guinea pigs and rabbits. They were removed after several days, the culture withdrawn and the virulency of the same tested. The results are shown in the following table:

Table of attempts to increase virulence of milk streptococci.

GUINEA-PIGS.

	Placed in guinea-pig.	Removed from guinea-pig.	Pathogenesis. —=not pathogenic. +=pathogenic.
A.....	January 4, 1909	January 9, 1909	—
B.....	January 4, 1909	January 9, 1909	—
D.....	January 4, 1909	January 11, 1909	—
G.....	January 4, 1909	January 11, 1909	—
1.....	January 4, 1909	January 9, 1909	—
2.....	January 4, 1909	January 9, 1909	—
3.....	January 4, 1909	January 9, 1909	—
6 es 27.....	January 4, 1909	January 9, 1909	—
7 s 14.....	January 4, 1909	January 9, 1909	—
7 k 25.....	January 4, 1909	January 11, 1909	—
7 d 25.....	January 4, 1909	January 11, 1909	—

RABBITS.

	Placed in rabbit.	Removed from rabbit.	Pathogenesis. —= not pathogenic. +=pathogenic.
A.....	February 14, 1909	February 21, 1909	—
B.....	February 14, 1909	February 21, 1909	—
D.....	February 14, 1909	February 21, 1909	—
G.....	February 14, 1909	February 21, 1909	—
1.....	February 14, 1909	February 21, 1909	—
2.....	February 14, 1909	February 21, 1909	—
3.....	February 14, 1909	February 21, 1909	—
6 es 27.....	February 14, 1909	February 21, 1909	—
7 s 14.....	February 14, 1909	February 21, 1909	—
7 d 25.....	February 14, 1909	February 21, 1909	—
7 k 25.....	February 14, 1909	February 21, 1909	—

In seven experiments, collodium sac cultures were allowed to remain in the abdominal cavities of guinea pigs for five days and in four experiments they were kept in that position for seven days. In attempts to increase their virulence by passage through rabbits, each of the eleven strains were retained in their bodies for seven days. An examination of hanging-drops made from the collodium sac cultures after removal from the abdominal cavities of the animals showed an abundant growth of the germs. The virulence of the germs was tested by inoculation into the ears of the rabbits.

The results of these experiments were negative in all cases. Contrary to this result Heinemann[9] has succeeded in raising the virulence of a number of strains of streptococcus lacticus by successive passage through rabbits to an equality with that of the streptococcus pyogenes.

The fact that their retention for a period of seven days in the bodies of both guinea pigs and rabbits without increasing their virulence would indicate that they are not easily endowed with pathogenicity and that their introduction into the digestive tract in milk would be harmful only by the chemical changes which they might effect in the contents of the intestines.

STREPTOCOCCUS LONGUS FROM OTHER SOURCES.

Streptococci are common in the feces of cattle and on the external surface of cows. In an examination of forty samples of fresh feces from the rectum of healthy cows, streptococcus longus was demonstrated in thirty-two. Probably a larger number could have been demonstrated by a more careful examination. In this regard Heinemann[4] says: "Streptococcus lacticus can be detected in cow feces, on the external surface of cows and in milk at all stages of subsequent handling." It seems quite probable that the contamination of the milk may be the result of their presence in the feces.

Streptococcus longus is almost universally present in the throat secretion of healthy individuals. Park found them in 83 per cent and states that he probably could have found them in some of the others by longer search.

With this class of organisms constantly present in the throats of healthy persons, and inducing in them no unfavorable conditions, it would seem unnecessary to concern ourselves with those which are introduced into our bodies in the milk supply. If the long streptococci normally present in the throat do not find conditions favorable for the development of virulence, apprehension of those in milk is in all probability unwarranted. Streptococci in milk from activity inflamed udders are of course excepted.

INGESTION EXPERIMENTS.

It is through ingestion of milk which contains these organisms that we expect to encounter danger. We should consider ingestion experiments of great importance in deciding their potency for harm. I have not carried out an extensive investigation in this regard and would suggest that further work be done before accepting any conclusions.

My experiments consisted in feeding two rabbits a large quantity of a strain of streptococcus longus which had been isolated from a sample of milk from a normal, healthy udder. A beef-tea culture was grown in the incubator forty-eight hours when it had become very cloudy and had some sediment. After

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shaking, 25 cubic centimeters of this culture were poured on a piece of bread and fed to the rabbits. No injurious influence could be detected in either case.

A causal relationship between these bacteria and summer diarrhea of children has been suggested. It has not been established that they are the agents in the milk which bring about this disturbance and it does not seem probable in consideration of the experiments just mentioned. It may be that they, in conjunction with other nonpathogenic bacteria, are detrimental at this time because of the increased fermentative action on the intestinal contents.

CONCLUSIONS.

1. The strains of milk streptococci commonly classed as streptococcus lacticus are indistinguishable culturally from streptococcus pyogenes.

2. The streptococci of this class comprise a large per cent of all streptococci present in milk from normal healthy udders.

3. Streptococcus lacticus does not acquire pathogenicity in the bodies of guinea pigs and rabbits when retained there for a period of seven days.

4. Streptococcus lacticus does not exert hemolytic action on blood agar.

5. Streptococci exactly similar to streptococcus lacticus are present in the throats of nearly all healthy individuals.

6. Streptococcus lacticus fed in large quantities to rabbits produces no ill effects.

7. Streptococcus lacticus in milk from healthy udders is not a source of danger to man.

8. Streptococci from diseased udders are often pathogenic.

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